

IN THE CLAIMS

Please amend Claims 1, 2, 4, 5, 7, 8, 12, 13, 17, 18, 29, and 34. In addition, please cancel claims 10, 11, and 22-27 and add new claims 37-46 as previously suggested in the corresponding office action response filed December 17, 2002.

1. (Currently Amended) A method of selection and/or identifying one or more protein affinity ligands, wherein the affinity ligands are antibodies, that bind to one or more proteins of interest, comprising the steps of:

(A) obtaining a real or theoretical mass spectrometry based characterization of the one or more proteins by either:

- i. Subjecting the one or more proteins to a mass spectrometry based characterization; or
- ii. Predicting the mass spectrometry based characterization from known data;

(F) (B) utilizing the one or more proteins either individually or as a mixture to:

- ~~j. i.~~ Generate one or more antibodies thereto by immunization; and/or
- ii. Select, using a single or multiple rounds of binding, one or more antibodies thereto;

(G) (C) screening to one or more antibodies generated in step B(i) and/or ~~the one~~

~~or more~~ multiple antibodies selected by step (B)(ii) by:

- ~~j. i.~~ adding a mixture of proteins or the one or more proteins individually ~~or as a mixture of proteins~~ to the one or more antibodies generated in step (B)(i) or the one or more antibodies selected in step (B)(ii), each antibody being used individually, and
- ii. after removing any proteins which have not bound, eluting the at least one protein has bound;

(H) (D) subjecting the at least one eluted protein to mass spectrometry based characterization; and

(H) (E) by comparing the mass spectrometry based characterization obtains in steps (A) and (D), selecting and/or identifying that at least one antibody that binds to the one or more proteins of interest.

2. (Currently Amended) A method as claimed in claim 1 wherein the one or more proteins of interest ~~are~~ have been previously resolved by 2D electrophoresis.

3. (Original) A method as claimed in claims 1 or 2 wherein between steps (B) and (C) the antibodies obtained in step (B)(i.) are cloned.

4. (Currently Amended) A method as claimed in claim 1 wherein the one ~~of~~ or more proteins of interest are present in a mixture of proteins.

5. (Currently Amended) A method as claimed in claim 1 wherein the method is a ~~shotgun~~ method for selecting and identifying protein affinity ligands to a plurality of proteins.

6. (Withdrawn)

7. (Currently Amended) A method as claimed in claim 1 wherein the antibodies optionally generated in step (B)(i) are immobilized on a support comprising nitrocellulose or PVDF.

8. (Currently Amended) A method as claimed in claim 7 wherein the support upon which the antibodies are immobilised and the nitrocellulose or PVDF are treated with an oligosaccharide or polyvinylpyrrolidine solution to block any remaining binding sites.

9. (Original) A method as claimed in claim 8 wherein the oligosaccharide is ficoll.

10. (Cancelled)

11. (Cancelled)

12. (Currently Amended) A method generating monoclonal antibodies to one or more targeted proteins comprising the steps of:

- (a) resolving a complex protein mixture;
- (b) subjecting the resolved protein(s) to peptide mass fingerprinting to obtain a peptide mass profile or obtain a theoretical peptide mass profile;
- (c) utilizing one or more of the resolved proteins to generate one or more monoclonal antibodies thereto;
- (d) adding the or another complex protein mixture to the one or more monoclonal antibodies generated in Step (c), to select those proteins which bind the one or more monoclonal antibodies, and subjecting the selected proteins(s) to peptide mass fingerprinting to obtain a peptide mass profile;
- (e) comparing the peptide mass profiles obtained in steps (b) and (d); and
- (f) selecting one or more monoclonal antibodies ~~hybridoma clones~~ of interest.

13. (Currently Amended) A method of generating an antibody library comprising the steps of:

- Ed*
- (a) resolving a complex protein mixture and subjecting the resolved protein(s) to peptide mass finger printing to obtain a peptide mass profile; or
 - (b) obtaining a theoretical peptide mass profile for a protein which is sought;
 - (c) utilizing the or the other complex protein mixture to generate one or more monoclonal antibodies thereto;
 - (d) adding the one or the other complex protein mixture to the one or more monoclonal antibodies generated in Step (c) to select those proteins which bind the one or more monoclonal antibodies, and subjecting the selected protein(s) to peptide mass fingerprinting to obtain a peptide mass profile;
 - (e) comparing the peptide mass profiles obtained in steps (a or b) and (d); and
 - (f) identifying the monoclonal antibodies of ~~potential~~ interest ~~for a monoclonal antibody library.~~
-

14. (Withdrawn)

15. (Previously Amended) A method of screening an antibody to a protein characterized in that the antibody is generated or selected using an impure protein or a complex protein mixture and then identified by comparing a mass spectrometry based characterization obtained from the protein/proteins for which it is specific with that of a mass spectrometry based characterization which is theoretical for said protein/proteins or is obtained from the impure protein or complex protein mixture.

16. (Previously Amended) A method of selecting an antibody specific to a given peptide characterized in that the antibody is selected by comparing a mass spectrometry based characterization of the protein/proteins released from the antibody to which it binds with a mass spectrometry based characterization which is theoretical for said protein/proteins or is obtained from the known protein.

17. (Currently Amended) A method as claimed in claims 1, 2, 7, 8, or 9, ~~10, 19, 20, 21, 22, 23, 24, 25, 26, or 27~~ wherein the mass spectrometry based characterization ~~peptide mass fingerprint~~ is obtained by mass spectrometry.

18. (Currently Amended) A method as claimed in claims 1, 2, 7, 8, or 9, ~~10, 19, 20, 21, 22, 23, 24, 25, 26, or 27~~ further comprising the use of automated well plate handling technology and automated high-throughput mass spectrometry.

19. (Previously Added) A method as claimed in claim 2 wherein the antibodies generated in step (B)(i.) are immobilized on a support comprising nitrocellulose or PVDF.

20. (Previously Added) A method as claimed in claim 19 wherein the support upon which the antibodies are immobilized are treated with an oligosaccharide or polyvinylpyrrolidine solution to block any remaining binding sites.

21. (Previously Added) A method as claimed in claim 20 wherein the oligosaccharide is ficoll.

22-27. (Cancelled)

28. (Previously Added) A method as claimed in claim 2 wherein the one or more proteins of interest are present in a mixture of proteins.

29. (Currently Amended) A method as claimed in claim 2 wherein the method is ~~shotgun~~ method for selecting and identifying protein affinity ligands to a plurality of proteins.

30. (Withdrawn)

31. (Previously Added) The method of claim 1 wherein said mass spectrometry based characterization is a peptide mass fingerprint.

32. (Previously Added) The method of claim 15 wherein said mass spectrometry based characterization is a peptide mass fingerprint.

33. (Previously Added) The method of claim 16 wherein said mass spectrometry based characterization is a peptide mass fingerprint.

34. (Currently Amended) A method of selecting and/or identifying at least one antibody which binds at least one protein of interest, comprising the steps of:

- (A) obtaining a theoretical ~~pre-selected~~ mass spectrometry-based characterization of a target protein ~~to serve as a reference standard~~;

34. (E) providing an antibody which selectively binds to said target protein;
- (C) isolating and collecting said target protein through affinity binding with said antibody;
- (D) analyzing said collected target protein for said pre-selected mass spectrometry-based characterization; and
- (E) comparing the mass spectrometry-based characterization obtained in step (D) with the theoretical mass spectrometry-based characterization ~~reference standard~~ of step (A).
-

35. (Previously Added) The method of selecting and/or identifying at least one antibody which binds at least one protein of interest recited in claim 34 wherein said mass spectrometry-based characterization is a peptide mass fingerprint.

36. (Previously Added) The method claim 35, further comprising the step of obtaining an additional mass spectrometry-based characterization in addition to said peptide mass fingerprint.

37. (New) A method as claimed in claim 1 wherein an eluting agent is further provided for eluting protein from antibody-protein complexes.

38. (New) A method as claimed in claim 37 wherein the eluting agent is formic acid.

39. (New) A method as claimed in claim 8 wherein an eluting agent is further provided for eluting protein from antibody-protein complexes.

40. (New) A method as claimed in claim 39 wherein the eluting agent is formic acid.
41. (New) A method as claimed in claim 19 wherein an eluting agent is further provided for eluting protein from antibody-protein complexes.
42. (New) A method as claimed in claim 41 wherein the eluting agent is formic acid.
43. (New) A method as claimed in claim 20 wherein an eluting agent is further provided for eluting protein from antibody-protein complexes.
44. (New) A method as claimed in claim 43 wherein the eluting agent is formic acid.
45. (New) A method as claimed in claim 21 wherein an eluting agent is further provided for eluting protein from antibody-protein complexes.
46. (New) A method as claimed in claim 45 wherein the eluting agent is formic acid.
-